Journal of Ethnopharmacology 137 (2011) 1215–1220

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Wound repair and anti-inflammatory potential of essential oils from cones of Pinaceae: Preclinical experimental research in animal models

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ARTICLE INFO

Article history:
Received 25 April 2011
Received in revised form 18 July 2011
Accepted 19 July 2011
Available online 26 July 2011

Keywords:
Abies
Cedrus
Picea
Pinaceae
Excision
Incision
Tensiometer
Wound healing
Anti-inflammatory
Medicinal plant

ABSTRACT

Ethnopharmacological relevance: Ethnobotanical surveys revealed that *Abies bornmulleriana*, *Abies cilicica*, *Abies nordmanniana* and *Cedrus libani* have been used to promote wound healing in Turkish folk medicine. Four different fir species (*Abies cilicica* subsp. *cilicica*, *Abies nordmanniana* subsp. *bornmulleriana*, *Abies nordmanniana* subsp. *equi-trojani*, and *Abies nordmanniana* subsp. *nordmanniana*), *Cedrus libani* and *Picea orientalis* were assessed for their *in vivo* wound healing and anti-inflammatory activities.
Materials and methods: The essential oils from six different coniferous cones were used. *In vivo* wound healing activity of the plants was evaluated by linear incision and circular excision experimental wound models subsequently histopathological analysis. The healing potential was comparatively assessed with a reference ointment Madecassol®, which contains 1% extract of Centella asiatica. Additionally acetic acid-induced capillary permeability test was used for the oils' anti-inflammatory activity.
Results: The essential oils from *Cedrus libani* and *Abies cilicica* subsp. *cilicica* demonstrated the highest activities on the both wound models. Moreover, the oil from *Abies nordmanniana* subsp. *bornmulleriana* was found generally highly effective. On the other hand, the rest of the species did not show any remarkable wound healing effect. Results of the present study support the continued and expanded utilization of these plant species employed in Turkish folk medicine.
Conclusion: The experimental study revealed that *Cedrus libani* and *Abies cilicica* subsp. *cilicica* display remarkable wound healing and anti-inflammatory activities.

1. Introduction

The family Pinaceae is a member of the coniferous, and includes many of the well-known conifers of commercial importance such as cedars, firs, hemlocks, larches, pines and spruces. It is found in most of the Northern Hemisphere with the majority of the species in temperate climates but ranging from sub arctic to tropical (Kurose et al., 2007).

According to literature surveys, it was reported that *Abies bornmulleriana*, *Abies cilicica* and *Abies nordmanniana* have been used to heal wounds, treat vascular diseases, gastric ulcers, bronchitis and common cold. For wound healing resin from *Abies* species is boiled together with grated root of *Saliva aethiopsis*, butter and beeswax and the mixture is then condensed into an ointment form. It is applied every day on cuts and other wounds for 5–10 days. Tar, obtained from *Cedrus libani* has been used for wound healing, against abdominal pain and rheumatism (Fujita et al., 1995; Yesilada et al., 1995; Sezik et al., 1997).

Several studies have been previously reported for Pinaceae. The chemical constituents are mostly terpenoids, especially, triterpenoids, flavonoids, lignans, phenols, steroids, fatty acids, and fatty alcohols (Yang et al., 2008a; Loizzo et al., 2008). The crude extracts and metabolites have been found to possess various bioactivities including insect juvenile hormone, antitumor, antiulcerogenic, anti-inflammatory, antihypertensive, antitussive, antimicrobial and central nervous system activities (Yang et al., 2008b). Particularly *Abies cilicia* and *Cedrus libani* extracts were shown to possess antimicrobial activity against *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Mycobacterium smegmatis*, *Proteus vulgaris*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Candida albicans*, *Candida tropicalis* and *Penicillium italicum* (Digrak et al., 1999).

The aim of the present study is to evaluate the folkloric information on wound healing activity of the essential oils from *Abies*, *Cedrus* and *Picea* species from the family Pinaceae by means of *in vivo* circular excision and linear incision wound models and anti-inflammatory activity.

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0378-8741/$ – see front matter
doi:10.1016/j.jep.2011.07.046

2. Materials and methods

2.1. Plant material

Six different coniferous cones were used in this study. Four different fir species (*Abies cilicica* subsp. *cilicica*, *Abies nordmanniana* subsp. *bornmulleriana*, *Abies nordmanniana* subsp. *equi-trojani*, *Abies nordmanniana* subsp. *nordmanniana*), *Cedrus libani* and *Picea orientalis* were collected and authenticated by Dr Barbaros Yaman, Faculty of Forestry at Bartin University, Bartin, Turkey. Voucher specimens of these species were deposited at the Herbarium of Faculty of Foresty as BOF 306 (*Abies nordmanniana* subsp. *bornmulleriana*), BOF 307 (*Abies nordmanniana* subsp. *nordmanniana*), BOF 308 (*Abies nordmanniana* subsp. *equi-trojani*), BOF 309 (*Abies cilicica* subsp. *cilicica*), BOF 310 (*Cedrus libani*), and BOF 311 (*Picea orientalis*). Approximately 2 kg of cones were collected for each species from their growth sites just at the time of maturity also packed tightly in plastic bags and stored in −24 °C until the laboratory studies (Tumen et al., 2010a,b). Species names, sampling site, collection date, climate zone, and altitude of all specimens are listed in Table 1.

2.2. Hydrodistillation

The essential oils of each sample were obtained by hydrodistillation with a Clevenger apparatus (ILDAM CAM Ltd., Ankara, Turkey) using 500 g (partially crushed) of fresh cones. The obtained oils were collected for 5 h and subsequently dried over anhydrous sodium sulphate and under refrigeration in a sealed vial until analyzed and tested (Tunalier et al., 2002; Tumen and Reunanen, 2010; Tumen et al., 2010c).

2.3. Biological activity tests

2.3.1. Animals

Male, Sprague-Dawley rats (160–180 g) and Swiss albino mice (20–25 g) were purchased from the animal breeding laboratory of Saki Yenilli (Ankara, Turkey).

The animals were left for 3 days at room conditions for acclimatization. They were maintained on standard pellet diet and water *ad libitum* throughout the experiment. A minimum of six animals were used in each group. The study was permitted by the Institutional Animal Ethics Committee (Gazi University Ethical Council Project Number: G.U.ET-08.037) and was performed according to the international rules considering the animal experiments and biodiversity right.

2.3.2. Preparation of test samples for bioassay

Incision and excision wound models were used to evaluate the wound healing activity. For the *in vivo* wound models, test samples were prepared in an ointment base (vehicle) consisting of glycol stearate, 1,2-propylene glycol, liquid paraffin (3:6:1) in 1% concentration. 0.5 g of each test ointment was applied topically on the wounded site immediately after wound was created by a surgical blade.

The animals of the vehicle group were treated with the ointment base only, whereas the animals of the reference drug group were treated with 0.5 g of Madecassol® (Bayer, 00001199). Madecassol contains 1% extract of *Centella asiatica*.

For the assessment of anti-inflammatory activity, test samples were given orally to test animals after suspending in a mixture of distilled H_2O and 1% Tween 80. The control group animals received the same experimental handling as those of the test groups except the drug treatment was replaced with appropriate volumes of dosing vehicle. Indomethacin (10 mg/kg) in 1% Tween 80 was used as reference drug.

2.3.3. Wound healing activity

2.3.3.1. Linear incision wound model. Animals, seven rats in each group, were anaesthetized with 0.15 cm^3 Ketalar® (Shetty et al., 2006), the hairs on the dorsal part of the rats were shaved and cleaned with 70% alcohol. Two 5-cm-length linear-paravertebral incisions were made with a sterile blade through the shaved skin at the distance of 1.5 cm from the dorsal midline on each side. Three surgical sutures were placed each 1 cm apart.

The ointments prepared with test samples, the reference drug (Madecassol®) or ointment base [glycol stearate:propylene glycol:liquid parafin (3:6:1)] were topically applied on the dorsal wounds in each group of animals once daily throughout 9 days. All the sutures were removed on the last day and tensile strength of previously wounded and treated skin was measured using a tensiometer (Zwick/Roell Z0.5, Germany) (Suguna et al., 2002; Lodhi et al., 2006).

2.3.3.2. Circular excision wound model. This model was used to monitor wound contraction and wound closure time. Each group of animals (seven animals in each) was anaesthetized by 0.01 cm^3 Ketalar®. The back hairs of the mice were depilated by shaving. The circular wound was created on the dorsal interscapular region of each animal by excising the skin with a 5 mm biopsy punch (Nopa instruments, Germany); wounds were left open (Tramontina et al., 2002). Test samples, the reference drug (Madecassol®, Bayer) and the vehicle ointments were applied topically once a day till the wound was completely healed. The progressive changes in wound area were monitored by a camera (Fuji, S20 Pro, Japan) every two days. Later on, wound area was evaluated using AutoCAD program. Wound contraction was calculated as percentage of the reduction in wounded area. A specimen sample of tissue was isolated from the healed skin of each group of mice for the histopathological examination (Sadaf et al., 2006).

2.3.4. Histopathology

The skin specimens from each group were collected at the end of the experiment. Samples were fixed in 10% buffered formalin, processed and blocked with paraffin and then sectioned into 5 μm sections and stained with hematoxylin and eosin (HE) and Van Gieson (VG) stains. The tissues were examined by light microscope (Olympus CX41 attached Kameram® Digital Image Analyze System) and graded as mild (+), moderate (++) and severe (+++) for epidermal or dermal re-modeling. Re-epithelization or ulcus in epidermis; fibroblast proliferation, mononuclear and/or polymorphonuclear cells, neo-vascularization and collagen depositions in dermis were analyzed to score the epidermal or dermal re-modeling. Van Gieson stained sections were analyzed for collagen deposition. At the end of the examination, all the wound healing processes were combined and staged for wound healing phases as inflammation, proliferation, and re-modeling in all groups.

2.3.5. Anti-inflammatory activity

2.3.5.1. Acetic acid-induced increase in capillary permeability. Effect of the test samples on the increased vascular permeability induced by acetic acid in mice was determined according to Whittle method (Whittle, 1964) with some modifications (Yesilada and Küpeli, 2007). Each test sample was administered orally to a group of 10 mice in 0.2 ml/20 g body weight. Thirty minutes after the administration, tail of each animal was injected with 0.1 ml of 4% Evans blue in saline solution (i.v.) and waited for 10 min. Then, 0.4 ml of 0.5% (v/v) AcOH was injected i.p. After 20 min incubation, the mice were killed by dislocation of the neck, and the viscera were exposed and irrigated with distilled water, which was then poured into 10 ml volumetric flasks through glass wool. Each flask was made up to 10 ml with distilled water, 0.1 ml of 0.1 N NaOH solution was added to the flask, and the absorption of the final solution was measured

Table 1
Sampling site, climate zone, date and altitude of the analyzed tree species.

Species	Sampling site	Climate zone	Collection date	Altitude (m)
Abies cilicica subsp. *cilicica*	Adana, Turkey	Mediterranean	May, 2010	600
Abies nordmanniana subsp. *bornmulleriana*	Bartin, Turkey	Temperate	October, 2010	1000
Abies nordmanniana subsp. *equi-trojani*	Balikesir, Turkey	Mediterranean	October, 2010	650
Abies nordmanniana subsp. *nordmanniana*	Trabzon, Turkey	Temperate	October, 2010	950
Cedrus libani	Adana, Turkey	Mediterranean	May, 2010	1100
Picea orientalis	Trabzon, Turkey	Temperate	October, 2010	900

at 590 nm (Beckmann Dual Spectrometer; Beckman, Fullerton, CA, USA). A mixture of distilled water and 0.5% CMC was given orally to control animals, and they were treated in the same manner as described above.

2.3.6. Statistical analysis of the data

The data on percentage anti-inflammatory and wound healing was statistically analyzed using one-way analysis of variance (ANOVA). The values of $p \leq 0.05$ were considered statistically significant.

Histopathologic data were considered to be nonparametric; therefore, no statistical tests were performed.

3. Results and discussion

Wound is an injury resulting in an opening or breaking of the skin. Wound healing is the process of repair which is fundamentally a connective tissue response. It is a multifarious procedure comprised of systematical processes of events which repair the damaged tissue partially or completely. The process can be broadly categorized into three stages; inflammatory phase (consisting the establishment of homeostasis and inflammation); proliferative phase (consisting of granulation, contraction and epithelialization) and finally the remodelling phase which ultimately determines the strength and appearance of the healed tissue (Kondo, 2007).

In this study, wound healing and anti-inflammatory activities of the essential oils from *Abies nordmanniana* subsp. *nordmanniana*, *Abies nordmanniana* subsp. *bornmulleriana*, *Abies nordmanniana* subsp. *equi-trojani*, *Abies cilicica* subsp. *cilicica*, *Cedrus libani* and *Picea orientalis* were assessed. For the evaluation of the wound healing activity, the oils were mixed with glycol stearate, 1,2-propylene glycol, liquid paraffin (3:6:1) in 1% concentration. Linear incision using tensiometer and circular excision wound models were employed for this activity assessment. More to the point, skin samples were also evaluated histopathologically. The experimental results are given in Tables 2–5. The study design of all these experiments is summarized in Table 6.

As shown in Table 2, topical application of the ointment prepared with the oil from *Cedrus libani*, oil from *Cedrus libani* and *Abies cilicica* subsp. *cilicica* onto the incised wounds demonstrated the best wound tensile strength by the highest values of 39.7% and 33.1% ($p < 0.01$) on day 10, respectively. Moreover, the oil obtained from *Abies nordmanniana* subsp. *bornmulleriana* was found generally highly effective (26.7%, $p < 0.05$) in linear incision wound model. On the other hand, the rest of the species did not show any remarkable wound healing activity.

The contraction values of the progression healing of wounds on circular excision wound model for vehicle, negative control, essential oils and reference drug treated groups were shown in Table 3. The essential oils from *Cedrus libani*, *Abies cilicica* subsp. *cilicica* and *Abies nordmanniana* subsp. *bornmulleriana* were found to have wound healing potential, while the vehicle and negative control groups and the other essential oil ointments showed no statistically significant wound healing activity. The wound contractions were 35.95% ($p < 0.05$) and 59.06% ($p < 0.001$) for *Cedrus libani*, 39.10% ($p < 0.05$) and 45.61% ($p < 0.01$) for *Abies cilicica* subsp. *cilicica*, and 20.44% and 37.43% ($p < 0.01$) for *Abies nordmanniana* subsp. *bornmulleriana* treated group on days 8 and 12, which were compared to reference drug Madecassol® [56.29% ($p < 0.01$)–100% ($p < 0.001$)].

Histopathological evaluation, scores and stages are summarized and presented in Table 5. For demonstrating of wound healing process, representative figures (Fig. 1), which stained with HE and VG, were also added. Phases in wound healing processes with varying degree were observed within the experimental groups. The re-modeling, especially, re-epithelization were detected, respectively, in the reference, *Cedrus libani*, *Abies cilicica* subsp. *cilicica*, *Abies nordmanniana* subsp. *bornmulleriana* and *Abies nordmanniana* subsp. *nordmanniana* groups and particularly in *Abies nordmanniana* subsp. *equi-trojani* ointment treated groups. The *Picea orientalis*, vehicle and negative control groups showed delayed wound healing processes compared to first groups. As an evidence of delaying, wound associated tissue debris are still remains in the dermal tissues of the last two groups.

The effect of the oils on the inflammatory phase of wound healing was examined using the method of Whittle, based on the inhibition of acetic acid induced capillary permeability. A dose-dependent inhibitory activity was observed for the oil from *Abies cilicica* subsp. *cilicica* and *Cedrus libani* at the dose of 200 mg/kg with the highest inhibitory value of 29.8% and 30.5% ($p < 0.01$), respectively (Table 4).

According to previous studies monoterpene type constituents were reported for *Abies*, *Cedrus* and *Picea* essential oils (Tumen et al., 2010a). Early reports indicated that essential oil components, especially monoterpenes, have multiple pharmacological effects (Edris, 2007). Some specific studies show that monoterpenes are cytotoxic, lipophilic, bactericidal, fungicidal, insecticidal, acting against osteoclasts, anticarcinogenic, pesticidal, antioxidant, anti-inflammatory, analgesic and sedative (Takayama et al., 2011). Limonene is an important monoterpene and has a role in wound healing (Adams and Thrash, 2010). In a model of chronic skin inflammation, the inventors have also shown a significant effect of limonene and per-

Table 2
Effect of the essential oils from *Abies cilicica* subsp. *cilicica*, *Abies nordmanniana* subsp. *bornmulleriana*, *Abies nordmanniana* subsp. *equi-trojani*, *Abies nordmanniana* subsp. *nordmanniana*, *Cedrus libani* and *Picea orientalis* on linear incision wound model.

Material	Statistical mean ± S.E.M.	Tensile strength (%)
Vehicle	15.42 ± 2.11	2.2
Negative control	15.09 ± 2.40	–
Abies cilicica subsp. *cilicica*	20.52 ± 1.21	33.1**
Abies nordmanniana subsp. *bornmulleriana*	19.54 ± 1.53	26.7*
Abies nordmanniana subsp. *equi-trojani*	17.68 ± 2.09	14.7
Abies nordmanniana subsp. *nordmanniana*	18.29 ± 1.37	18.6
Cedrus libani	21.54 ± 1.09	39.7**
Picea orientalis	17.04 ± 2.12	9.5
Madecassol®	24.27 ± 1.02	57.4***

Percentage of tensile strength values: vehicle group was compared to negative control group; the oils and the reference material were compared to vehicle group; S.E.M., standard error meaning.

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 3
Effect of the essential oils from *Abies cilicica* subsp. *cilicica*, *Abies nordmanniana* subsp. *bornmulleriana*, *Abies nordmanniana* subsp. *equi-trojani*, *Abies nordmanniana* subsp. *nordmanniana*, *Cedrus libani* and *Picea orientalis* on circular excision wound model.

Material	Wound area ± S.E.M. (contraction %)						
	0	2	4	6	8	10	12
Vehicle	19.48 ± 2.14	18.22 ± 2.12 (4.11)	15.26 ± 1.98 (3.78)	13.48 ± 1.74 (3.92)	9.54 ± 1.42 (13.43)	6.77 ± 1.05 (5.71)	3.42 ± 0.73 (13.64)
Negative control	19.57 ± 2.01	19.00 ± 2.16	15.86 ± 2.04	14.03 ± 1.88	11.02 ± 1.89	7.18 ± 1.28	3.96 ± 1.09
Abies cilicica subsp. *cilicica*	19.45 ± 2.23	15.05 ± 1.75 (17.40)	12.97 ± 1.84 (15.01)	9.85 ± 1.28 (26.93)	5.81 ± 1.16 (39.10)*	4.00 ± 0.57 (40.92)**	1.86 ± 0.29 (45.61)**
Abies nordmanniana subsp. *bornmulleriana*	19.49 ± 2.30	17.20 ± 1.70 (5.60)	14.14 ± 1.77 (7.34)	11.12 ± 1.63 (17.51)	7.59 ± 1.22 (20.44)	5.10 ± 0.85 (24.67)	2.14 ± 0.56 (37.43)**
Abies nordmanniana subsp. *equi-trojani*	20.01 ± 2.74	17.56 ± 1.59 (3.62)	14.45 ± 1.88 (5.31)	13.75 ± 1.97 –	8.12 ± 1.60 (14.88)	5.44 ± 1.26 (19.65)	2.83 ± 0.50 (17.25)
Abies nordmanniana subsp. *nordmanniana*	19.75 ± 2.35	17.19 ± 2.01 (5.65)	15.20 ± 1.83 (0.39)	12.24 ± 1.94 (9.20)	9.26 ± 1.70 (2.9)	6.08 ± 1.13 (10.19)	3.21 ± 0.65 (6.14)
Cedrus libani	19.91 ± 2.37	16.95 ± 2.24 (6.97)	13.93 ± 1.79 (8.72)	10.01 ± 1.55 (25.74)	6.11 ± 1.15 (35.95)*	3.95 ± 0.51 (41.65)**	1.40 ± 0.14 (59.06)***
Picea orientalis	19.62 ± 2.45	18.15 ± 2.17 (0.38)	15.38 ± 1.93 –	13.50 ± 1.49 –	9.11 ± 1.56 (4.51)	6.70 ± 1.56 (1.03)	3.02 ± 0.96 (11.70)
Madecassol®	19.52 ± 2.19	14.93 ± 1.76 (18.06)	12.10 ± 1.58 (20.71)	8.08 ± 1.10 (40.06)**	4.17 ± 0.95 (56.29)**	1.59 ± 0.36 (76.51)***	0.00 ± 0.00 (100.00)***

Percentage of contraction values: vehicle group was compared to negative control group; the oils and the reference material were compared to vehicle group; S.E.M., standard error meaning.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 4
Inhibitory effect of the essential oils from *Abies cilicica* subsp. *cilicica*, *Abies nordmanniana* subsp. *bornmulleriana*, *Abies nordmanniana* subsp. *equi-trojani*, *Abies nordmanniana* subsp. *nordmanniana*, *Cedrus libani* and *Picea orientalis* on acetic acid-induced increased capillary permeability.

Material	Dose (mg/kg)	Evans blue concentration (μg/ml) ± S.E.M.	Inhibition (%)
Control		12.24 ± 0.92	
Abies cilicica subsp. *cilicica*	100 200	9.23 ± 0.46 8.59 ± 0.43	24.6* 29.8**
Abies nordmanniana subsp. *bornmulleriana*	100 200	10.15 ± 0.48 9.76 ± 0.47	17.1 20.3
Abies nordmanniana subsp. *equi-trojani*	100 200	12.03 ± 0.89 11.28 ± 0.90	1.7 7.8
Abies nordmanniana subsp. *nordmanniana*	100 200	10.56 ± 0.98 9.94 ± 0.80	13.7 18.8
Cedrus libani	100 200	9.74 ± 0.65 8.51 ± 0.27	20.4 30.5**
Picea orientalis	100 200	13.06 ± 0.96 11.99 ± 0.84	– 2.0
Indomethacin	10	6.16 ± 0.21	49.7***

S.E.M., standard error meaning.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 5
Wound healing processes and healing phases of the vehicle, negative control, oils and Madecassol® administered animals.

Groups	Wound healing processes								Healing phases		
	S	U	RE	FP	CD	MNC	PMN	NV	I	P	R
Vehicle	++/+++	+++	–	++/+++	++/+++	++	++/+++	++	++/+++	++/+++	–
Negative control	+++	+++	–	++	+/++	+	++/+++	+/++	++/+++	++	–
Abies cilicica subsp. *cilicica*	+	–	++	++	++	++	+	+/++	+/++	++	++
Abies nordmanniana subsp. *bornmulleriana*	+/++	+	+/++	++	++	+/++	+	++	+/++	++	+/++
Abies nordmanniana subsp. *equi-trojani*	++/+++	++	–/+	++	++	+/++	++	++/+++	++	++/+++	–/+
Abies nordmanniana subsp. *nordmanniana*	+/++	–/+	+/++	++	++	+/++	+	+/++	++	++	+/++
Cedrus libani	+	–	++	+/++	+/++	+/++	–/+	+/++	+	+/++	++
Picea orientalis	+++	+++	–	++/+++	++	++	++/+++	++/+++	++/+++	++/+++	–
Madecassol®	+	–	++	+/++	+/++	+/++	–/+	+/++	+	+/++	++

HE and VG stained sections were scored as mild (+), moderate (++) and severe (+++) for epidermal and/or dermal re-modeling. S, scab; U, ulcus; RE, re-epithelization; FP, fibroblast proliferation; CD, collagen depositions; MNC, mononuclear cells; PMN, polymorphonuclear cells; NV, neovascularization; I, inflammation phase; P, proliferation phase; R, re-modeling phase.

Table 6
Study design of the experimental models.

Main groups for all models	Experimental model	Animals in each group	Evaluation parameters	Time of the evaluation	Experiment duration	Investigations after the experiments
Negative control	Linear incision wound	7 rats	Tensile strength	10th day	10 days	Histopathological assessment
Vehicle Essential oils	Circular excision wound	7 mice	Wound contraction	Every alternate day	12 days	
Reference drug	Whittle	10 mice	Inhibition of acetic acid-induced increase in capillary permeability	1st hour	1 h	–

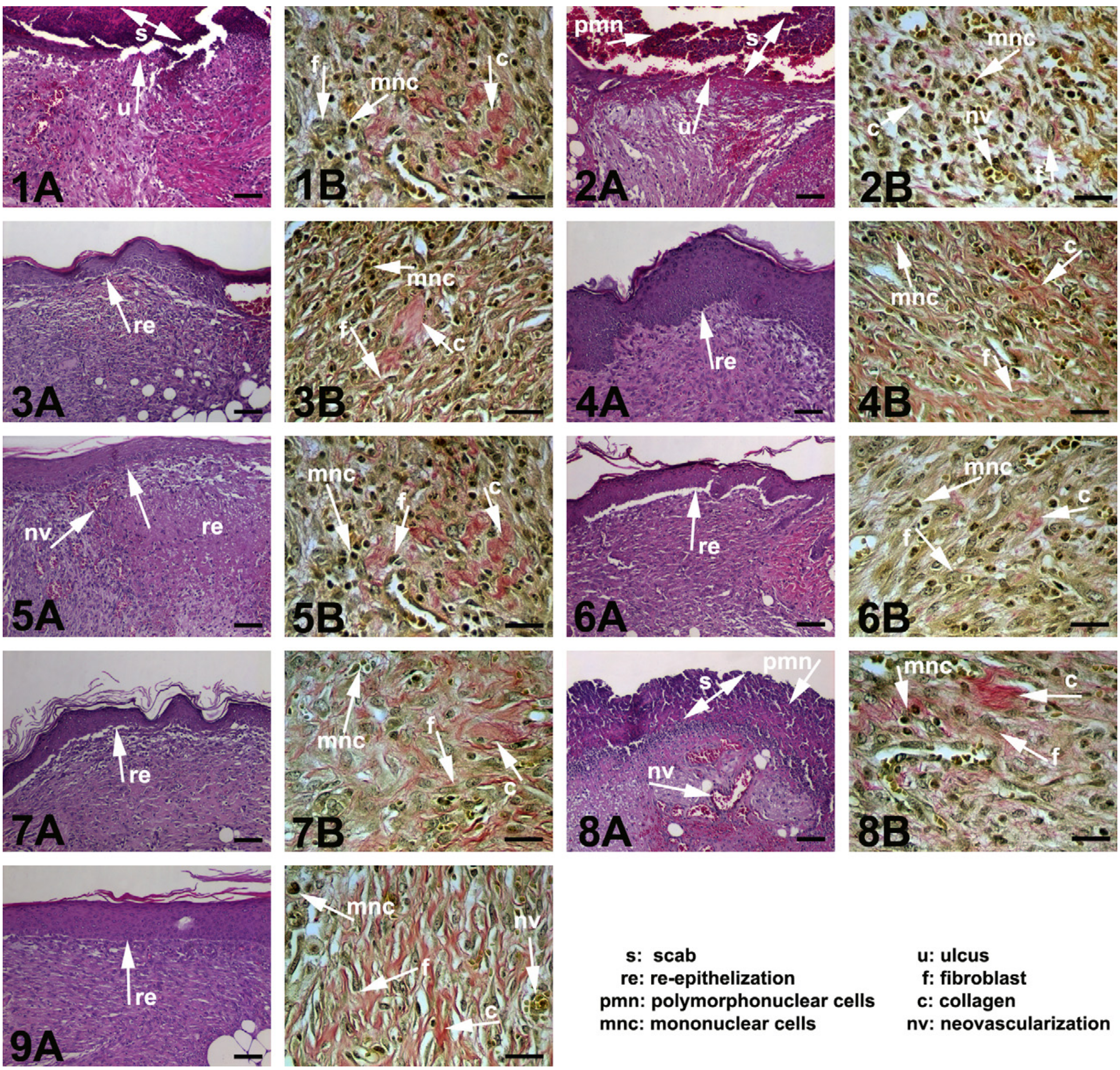

Fig. 1. Histopathological view of wound healing and epidermal/dermal re-modeling in the vehicle, negative control, oils and reference ointment Madecassol® administered animals. Skin sections show the hematoxylin and eosin (HE) stained epidermis and dermis in (A), and the dermis stained with Van Gieson (VG) in (B). The original magnification was 100× and the scale bars represent 120 μm for figures in (A), and the original magnification was 400× and the scale bars represent 40 μm for (B). Data are representative of 6 animal per group. (1) Vehicle group, 10-day-old wound tissue treated with only vehicle, (2) negative control group, 10-day-old wound tissue, untreated group, (3) *Abies cilicica* subsp. *cilicica* group, 10-day-old wound tissue treated with the essential oil of *Abies cilicica* subsp. *cilicica*, (4) *Abies nordmanniana* subsp. *bornmulleriana* group, 10-day-old wound tissue treated with essential oil of *Abies nordmanniana* subsp. *bornmulleriana*, (5) *Abies nordmanniana* subsp. *equi-trojani* group, 10-day-old wound tissue treated with essential oil of *Abies nordmanniana* subsp. *equi-trojani*, (6) *Abies nordmanniana* subsp. *nordmanniana* group, 10-day-old wound tissue treated with essential oil of *Abies nordmanniana* subsp. *nordmanniana*, (7) *Cedrus libani* group, 10-day-old wound tissue treated with essential oil of *Cedrus libani*, (8) *Picea orientalis* group, 10-day-old wound tissue treated with essential oil of *Picea orientalis*, and (9) reference group, 10-day-old wound tissue treated with Madecassol®.

illyl alcohol on skin repair and pro-inflammatory cytokines levels (Stanley et al., 1991). *Cedrus libani* was found to have the highest limonene content with 22.7% when compared to *Abies* and *Picea* sp. (Tumen et al., 2010a). Therefore, the highest wound healing activity could be attributed to its high limonene content.

Moreover *Abies* and *Cedrus* species are rich in water soluble sugar units and cellulose content (Kilic et al., 2010). These constituents were also proved to possess wound healing activities (Kossi et al., 2004).

4. Conclusion

According to experimental results, essential oils obtained from *Cedrus libani* and *Abies cilicica* subsp. *cilicica* were found to have better activity on the wound healing compared to the other oils and control groups. This might be due to the combined effect of the constituents present in the oils. Further studies are warranted to isolate active compounds and to elucidate their exact mechanism of action.

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